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(54) Title: RECOVERING A MONOSACCHARIDE FROM A SOLUTION USING A WEAKLY ACID CATION EXCHANGE RESIN FOR THE CHROMATOGRAPHIC SEPARATION

(57) Abstract: The present invention relates to a method for recovering a monosaccharide selected from the group consisting of rhamnose, arabinose, xylose and mixtures thereof from a solution containing the same by a multistep process using chromatographic separation comprising at least one step, where a weakly acid cation exchange resin is used for the chromatographic separation.

Recovering a monosaccharide from a solution using a weakly acid cation exchange resin for the chromatographic separation.

FIELD OF THE INVENTION

The present invention relates to a method comprising a multistep process for recovering rhamnose and optionally arabinose. More particularly the present invention relates to the use of a weakly acid cation exchange resin in a chromatographic column in a multistep process.

BACKGROUND OF THE INVENTION

US Patent 2 684 331 discloses a method for separating chromatographically from one another two or more substances having widely different ionization constants and at least one of the substances undergoes considerable ionization in a dilute aqueous solution thereof. However, the method has not been used for separating sugars. The examples of the US Patent 2 684 331 describe separation of salts from organic solvents, e.g. sodium chloride from formaldehyde. The method comprises an ion exchange resin having an ion identical with an ion of highly ionized solute. The ion exchange resin is either a cation exchange resin having an acidic form or an anion exchange resin having a basic form. The cation exchange resin contains sulphonic acid groups. The anion exchange resin contains quaternary ammonium groups.

US Patent 2 911 362 described a method comprising a chromatographic separation process employing ion exchange resins for separating two or more water soluble organic compounds from one another in an aqueous medium in the absence of an ion exchange reaction, i.e. in the substantial absence of a chemical reaction involving an absorption of ions from the aqueous medium by the resin or the introduction of ions into the solution from the resin. According to said method the ion exchange resin can be either a cation exchange resin or an anion exchange resin. The cation exchange resin may contain either sulfonic acid groups or carboxylic acid groups. The anion exchange resin contains quaternay ammonium groups. However, the method has not been used for separating sugars.

Chromatographic separation has been used for recovering xylose from hydrolysates of natural materials such as birch wood, corn cobs and cotton seed hulls in a method described in U.S. Patent No. 4 075 406. The resin employed in the chromatographic separation is a strongly acid cation exchanger, i.e. sulfonated polystyrene cross-linked with divinyl benzene.. The use of a strongly acid cation exchanger for separating monosaccharides, e.g. xylose, from magnesium sulfite cooking liquor is also known from Finnish Patent Application No. 962 609. The chromatographic separation is carried out by using a simulated moving bed. However, the separation of certain monosaccharides by using strongly acid cation exchange resins has turned out to be difficult. For instance the separation of rhamnose from other carbohydrates with strongly acid cation exchange resins and strongly basic cation exchange resins has been possible by using solvents such as alcoholic solvents as eluents (see e.g. Samuelson O., Chromatography on ion exchange resins, J. Methods Carbohy. Chem. 6 (1972) 65 - 75). In the described system anhydro sugars, such as rhamnose, have a shorter retention time than most of the aldoses and ketoses. Water would be a preferred eluent, but the use of water has not, however, been described in this connection. The problem when using water is that the various monosaccharides, such as rhamnose, xylose and arabinose, have almost similar retention times, whereby the fractions will overlap.

The separation of carbohydrates, especially xylose by strongly acid cation exchangers has been practiced industrially but is complicated. The method presented in US Patent No. 5 998 607 has been used especially for separating xylose from magnesium spent liquor. The problem has been the unsufficient separation of xylose and xylonic acid and there is no suggestion of the use of a weakly acid cation exchange resin possibly giving a benefit for solving the problem. In the disclosed method the separation requires two steps. In the first step the cation exchange resin is used preferably in alkaline earth form, more preferably in Mg^{2+} form and in the second step the cation exchange resin is preferably in alkali-metal form (e.g. sodium). However, the separation of monosaccharides has also been found to be unsatisfactory since

all the other sugars elute at almost similar retention time with xylose. The pH used in the process low. The resin in a dival nt form seemed to separate the xylose more effectively than the resin in a monovalent form.

Anion exchange resins have been used for separating fructose from glucose. Y. Takasaki (Agr. Biol. Chem. 36 (1972) pages 2575 - 77) and B. Lindberg et al. (Carbohyd. Res. 5 (1967), pages 286 - 291) describe the use of an anion exchanger in bisulfite form for the separation of sugars. However, the use of anion exchange resins does not result in good xylose separation because xylose is overlapped by other sugars.

US patent 4 358 322 discloses a process for separating fructose from a feed mixture comprising fructose and glucose. The process comprises contacting the mixture with an adsorbent comprising aluminosilicate or zeolite.The adsorbent contains one or more selected cations at exchangable cation sites. The cations are selected from the group consisting of sodium, barium and strontium. The cationic pairs used in the cationic sites are selected from the group consisting of barium and potassium and barium and strontium.

U.S. Patent No. 5 084 104 discloses a method for the separation of xylose from a pentose-rich solution, e.g. birch wood. A chromatographic column which comprises a strongly basic anion exchange resin is used. The anion exchange resin is in sulfate form. Using this method xylose is retarded most strongly and the other monosaccharides are eluted faster.

A method for preparing of L-arabinose is known from the publication WO 99/57326 where the process is characterized by contacting plant fibers with an acid to hydrolyze the fibers under such conditions that the L-arabinose ingredients contained in the plant fibers are selectively obtained. U.S. Patent No. 4 880 919 discloses a process for separating arabinose from mixtures of monosaccharides containing arabinose and other aldopentoses and aldohexoses by adsorption on sulfonated polystyrene divinyl benzene crosslinked ion exchange resins exchanged with Ca^{2+} and NH_4^+ ions and desorpting the adsorbate with water. A process for producing crystalline L-arabinose is known from U.S. Patent No. 4 816 078.

The preparation of arabinose is also known from US Patent 4 664 718. In the described method arabinose is separated from a monosaccharide mixture containing also other aldopentoses and aldohexoses. The feed is contacted with with a calcium-Y-type or calcium-X-type zeolite and arabinose is adsorbed selectively. The desorption is conducted with water or ethanol.

Publication DE 3 545 107 describes a method for the preparation of rhamnose from arabic gum. A strongly acid cation exchange resin is used for the separation of sugars and rhamnose by adsorption with activated charcoal. Arabinose is also separated by this method.

Barker, S.A. et al (Carbohydrate Research, 26 (1973) 55 - 64) have described the use of poly(4-vinylbenzeneboronic acid) resins in the fractionation and interconversion of carbohydrates. In the method water is used as an eluent. The best yield of fructose was received when the pH was high. The resins have also been used to displace the pseudo equilibrium established in aqueous alkali between D-glucose, D-fructiose and D-mannose to yield D-fructose.

CA Patent No. 1 249 812 discloses a multistep process for the separation of sugars and lignosulphonates fron sulphite spent liquor. The process comprises the steps of (a) introducing sulphite spent liquor having a certain pH into a chromatographic column containing a resin in metal salt form, (b) eluting the column with water to obtain a substiantially sugar-free lignosulphonate-rich fraction and a sugar-rich fraction, (c) collecting the sugar-rich fraction for further purification, (d) adjusting the pH of the fraction to a certain level and introducing it to a second column containing a resin in monovalent metal salt form, and (e) eluting the sugar-rich material from the second column to obtain a sugar-rich fraction and a lignosulphonate-rich fraction. The process of said CA patent does not include the use of a weakly acid cation exchange resin for chromatographic separation.

A process for crystallizing xylose is known from Finnish Patent 97 625. In the process xylose is recovered by crystallization from the solutions

in which the xylose purity is relatively low. Especially the process concerns recovering xylose from biomass derived solutions.

When xylose is prepared by hydrolysing biomass derived xylose rich hemicellulose the mixture contains among xylose also glucose, galactose, rhamnose, mannose and arabinose. It also may contain acetic acid and uronic acids such as galacturonic acid and glucuronic acid. The hydrolysing acid and the uronic acid are generally easily removed by ion exclusion. However, it has been difficult to fractionate monosaccharide mixtures to their components.

Surprisingly it has been found that rhamnose and, if desired, arabinose can be effectively separated from carbohydrate streams by using weakly acid cation exchange resins. The order of elution seems to be, besides other factors, affected strongly by the hydrophobic/hydrophilic interaction between the carbohydrate and the resin. If the resin is in hydrophilic form, the most hydrophobic carbohydrate seems to elute first and the most hydrophilic last. For instance, the resin in H^+ form seems to be less hydrophilic than the resin in Na^+ form. The different elution order of components in a chromatographic column using a weakly acid cation exchange resin can be effectively used in the method of the present invention comprising a multistep process.

SUMMARY OF THE INVENTION

The above mentioned objects and others are accomplished by the present invention, which relates to a method for recovering a monosaccharide selected from the group consisting of rhamnose, arabinose, xylose and mixtures thereof from a solution containing at least two of said monosaccharides by a multistep process using chromatographic separation comprising at least one step, where a weakly acid cation exchange resin is used for the chromatographic separation. The method may preferably contain additional steps comprising the use of chromatographic columns containing strongly acid cation exchange resins, evaporation, crystallization, etc.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings are illusrative embodiments of the invention and are not meant to limit the scope of the invention as defined in the claims.

FIG. 1 is a graphical presentation of the elution profiles and pH according to Example 1.

FIG. 2 is a graphical presentation of the elution profiles and pH according to Example 2.

FIG. 3 is a graphical presentation of the elution profiles and pH according to Example 3.

FIG. 4 is a graphical presentation of the elution profiles and pH according to Example 4.

FIG. 5 is a graphical presentation of the elution profiles and pH according to Example 5.

FIG. 6 is a graphical presentation of the elution profiles and pH according to Example 6.

FIG. 7 is a graphical presentation of the elution profiles and pH according to Example 7.

FIG. 8 and 9 are schematic presentations of embodiments of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

According to the present invention a solution containing a monosaccharide selected from the group consisting of rhamnose, arabinose, xylose and mixtures thereof is subjected to a multistep process using chromatographic separation comprising at least one step, where a weakly acid cation exchange resin is used in a chromatographic column or part of it. The multistep process according to the invention may preferably comprise additional steps, such as steps using chromatographic columns containing strongly acid cation exchange resins, evaporation, crystallization, etc. in order to enhance the effective separation of the desired product. Suitable starting solutions are those obtained by hydrolyzing hemicellulose. In addition to rhamnose the starting solution preferably contains arabinose and possibly xylose. Such solutions

are for instance xylose process streams and side streams. In addition to rhamnose also other carbohydrates may be recovered by the method of the present invention. Such carbohydates are e.g. monosaccharides, such as arabinose, preferably L-arabinose, xylose, preferably D-xylose and mixtures thereof. The general opinion has been that an effective separation of the monosaccharides in question requires the use, for instance, of ion exclusion and size exclusion. The additional feature relating to the use of a weakly acid cation exchange resin is that if the resin is in hydrophilic form the most hydrophobic monosaccharide seems to be eluted first and the most hydrophilic monosaccharide is eluted last.The solution containig rhamnose treated may be a product obtained from the processing of hydrolysates or prehydrolysates of hemicellulose from hard wood and xylose containing biomass, e.g. solutions formed in paper and dissolving pulp processing, for example si-cooking or prehydrolysis of sa-cooking.

The chromatographic column used in the method of the present invention is filled with a weakly acid cation exchange resin, preferably an acrylic cation exchange resin having carboxylic functional groups. However, the resin can be other than an acrylic resin and the functional group can be other than a carboxylic group, e.g. another weak acid. Such an acrylic resin is preferably derived from methyl acrylate, ethyl acrylate, buthyl acrylate, methylmethacrylate or acrylonitrile or acrylic acids or mixtures thereof.. The resin may be crosslinked with a cross-linking agent, e.g. divinyl benzene (DVB). A suitable crosslinking degree is 1 to 20 % by weight, preferably 3 to 8 % by weight. The average particle size of the resin is normally 10 to 2000 µm, preferably 100 to 400 µm. The resin may be regenerated into H^+, Na^+, Mg^{2+} or Ca^{2+} form. However, also other ionic forms may be used.

The column is preferably eluted at temperatures from 10 to 95 °C, more preferably from 30 to 95 °C, more prefreably from 55 to 85 °C. It is known that a higher separation temperature decreases the viscosity and improves the separation performance.

The eluent used in the chromatographic separation according to the present inv ntion is either water, a solvent, e.g. an alcohol, or a mixture thereof. Preferably the eluent is water.

The carbohydrate solution to be fractioned is optionally filtrated before chromatographic separation, whereby the filtration may be carried out by using a pressure filter and diatomaceous earth as a filter aid. The pH of the feed solution is optionally adjusted to 1 to 10, preferably 2 to 10, more preferably 2 to 4 and 5 to 10. For instance when pH is high, i.e. 6 to 7, rhamnose is separated first before other more hydrophilic monosaccharides. After the pH has been adjusted the solution may be filtered. The dry substance of the feed solution is adjusted to an appropriate level before chromatographic separation.

A feeding device is used for feeding the solution to the column. The temperature of the column, feed solution and eluent is most preferably approximately 65 °C. This is accomplished by preheating the feed solution. The feed solution is eluted in the column by feeding water, for instance demineralized water or e.g. condensate or some other aqueous solution, alcohol or a mixture thereof into the column. The eluent may be pumped through a heat exchanger as well. The flow rate in the column is adjusted to an appropriate level. The fractions of the outcoming solutions are collected at suitable intervals and analyzed. The outcome from the column may be monitored by online instruments. The fractionated products, e.g. rhamnose and arabinose, may be isolated by crystallization afterwards or in the following step. Also recycle fractions collected from the other end of the column may be used in a way known per se.

It is clear for the person skilled in the art that the multistep process can be altered by reorganizing the order of the process units or by adding or removing some process units. The person skilled in the art may also add or alter the order of other separation, recovering and concentration units.

Further, it is possible to arrange two or more chromatographic columns in sequence wherein at least one column contains a weakly acid cation excgange resin, the other columns possibly containing a strongly acid cation exchange resin. Also simulated moving bed (SMB) systems may be used. The

simulated moving bed system can be either sequential or continuous. In a preferred embodiment of the inv ntion a first column containing a strongly acid cation exchange resin is connected to a second column containing a weakly acid cation exchange resin. Fractions obtained from the second column may be lead to one or more further columns containing either strongly acid or weakly acid cation exchange resins. Such an arrangment further improves the separation performance and increases the yields and purity of the products such as rhamnose, arabinose and xylose. Between the columns there are optionally additional process steps comprising, e.g. precipitation, filtration, crystalliza-tion, evaporation or some other concentration process steps or other known process units.

In the multistep process according to the present invention where a weakly acid cation exchange resin is used the elution order of rhamnose and other saccharides is advantageously different from the elution order obtained by using strongly basic resins in bisulfite form or sulfate form or using strongly acid cation exchange resins. One of the advantages relating to the present invention is that different elution order of the components in the chromatographic column is advantageously used in the method of the invention comprising a multistep process. One of the product fractions received is a rhamnose rich fraction, one is a xylose rich fraction, and one is a arabinose rich fraction. According to the multistep process of the present invention using a weakly acid cation exchange resin in a first step rhamnose is preferably eluted before the other monosaccharides, when the resin is in hydrophilic form. This allows rhamnose, and also the other carbohydrates, to be received in good yields with high purity. When the resin is in a more hydrophobic form, rhamnose is eluted in the back slope of the monosaccharide separation.

Figure 8 presents a schematic drawing where crystalline xylose is produced. The crystallization mother liquor is used in a multistep process for producing rhamnose, comprising at least one step using a weakly acid cation exchange resin.

Figure 9 shows a more detailed example of a multistep process for producing rhamnose. At first xylose is purified in the xylose process and a

xylose fraction is recovered. Also an arabinose fraction may be collected. The crystallization mother liquor of the xylose process is further purified by chromatographic separation. The resin may be a weakly acid or strongly acid cation exchange resin.The separation is continued by chromatographic separation and a rhamnose rich fraction is recovered. Again, a weakly acid or strongly acid cation exchange resin can be used. The rest of the outflow can be further separated using a strongly acid cation exchange resin and more xylose can be recovered. Also arabinose may be collected at this step.

Also the rhamnose rich fraction is further purified by using either a weakly or strongly acid cation exchange resin. However, at least one of the thre chromatographic separation steps for the rhamnose fraction is carried out by using a weakly acid cation exchange resin.

Rhamnose crystallization may be carried out after the last separation step. The product obtained is suitably rhamnose monohydrate.

The method according the present invention makes it possible to separate and recover rhamnose and also other products, such as rhamnose, arabinose and xylose, in good yields from solutions containing rhamnose, which has been very difficult by known methods using e.g. strongly acid cation exchange resins. One of the advantages achieved by the method of the present invention over the prior art is that the use of a weakly acid cation exchange resin allows the use of water as an eluent for efficient separation. Known methods using strongly acid cation exchange resins for efficient separation of carbohydrate_products of the type mentioned above always require that the eluent is a solvent, e.g. aqueous alcohol. However, when water is used as the eluent, the handling is easier, the costs are lower and the safety is higher. By using water as the sole eluent problems relating to e.g. storage and regeneration are avoided.

The following examples illustrate the present invention. The examples are not to be construed to limit the claims in any manner whatsoever.

Example 1

Chromatographic separation of xylose crystallization run-off with a H^+/Mg^{2+} -form resin

Xylose crystallization run-off which was beech wood based Mg-base si-cooking liquor was subjected to a chromatographic separation. The separation was performed in a laboratory chromatographic separation column as a batch process. The column with a diameter of 0,045 m was filled with an acrylic weakly acid cation exchange resin (Finex CA 12 GC™) manufactured by Finex Oy, Finland. The resin was an ethyl acrylate -based resin. The height of the resin bed was about 0,70 m. The cross-linkage degree of the resin was 6 % by weight DVB and the average particle size of the resin was 0,26 mm. The resin was regenerated into mainly H^+-form (94% by equivalent) and partly Mg^{2+}-form (6% by equivalent) and a feeding device was placed at the top of the resin bed. The temperature of the column and feed solution and eluent water was approximately 65°C. The flow rate in the column was adjusted to 4 ml/min.

The chromatographic separation was carried out as follows:

Step 1:

The dry substance of the feed solution was adjusted to 25 g dry substance in 100 g solution according to the refractive index (RI) of the solution. The pH of the feed solution was 3,5.

Step 2:

100 ml of preheated feed solution was pumped to the top of the resin bed.

Step 3:

The feed solution was eluted downwards in the column by feeding preheated ion-exchanged water to the top of the column.

Step 4:

10 ml samples of the outcoming solution were collected at 3 min intervals. The composition of the samples was analysed with Dionex HPLC equipment with pulsed electrochemical detector and CarboPac PA1™ anion exchange column (water and 0,2 M NaOH as eluents).

The resin gives a good separation of rhamnose and arabinose from other monosaccharides. Rhamnose and arabinose are eluted at the end of the profile. The pH of the effluent was between 3 and 4. The results are shown graphically in FIG. 1.

Example 2

Purification of L-rhamnose by chromatograhic separation

Xylose precipitation crystallization (the final run-off) mother liquor from birch wood based si-cooking was used as a starting material and was thus subjected to a chromatographic separation in a batch separation column.

The separation was performed in a pilot chromatographic separation column as a batch process. The whole equipment consisted of a feed tank, a feed pump, a heat exchanger, a chromatographic separation column, product containers, pipelines for input of feed solution as well as eluent water, pipelines for output and flow controlling equipments.

The column with a diameter of 0,225 m was filled with an acrylic weakly acid cation exchange resin (manufactured by Finex Ltd., Finland); the height of the resin bed was about 5,2 m. The degree of cross-linkage was 3 % by weight DVB and the average particle size was 0,34 mm. The resin was regenerated into sodium (Na^+) form and a feeding device was then placed at the top of the resin bed. The temperature of the column, feed solution and eluent water was 65 °C. The flow rate in the column was adjusted to 40 l/h.

The feed solution was pretreated first by filtration, which was done using a pressure filter and diatomaceous earth as filter aid. The feed solution was then heated to 65 °C and the pH was adjusted to pH 6,0 with sodium hydroxide solution, after which the solution was filtered.

Chromatographic separation was carried out as follows:

Step1:

The dry substance of the feed solution was adjusted to 35 g dry substance in 100 g of solution according to the refractive index (RI) of the solution.

Step2:

20 l of preheated feed solution was transferred to the top of the resin bed

Step 3:

The feed solution was eluted downwards in the column by feeding ion exchanged preheated water to the top of the column.

Step 4:

The density and conductivity of the outcoming solution were measured continuously and according to this information, the outcoming solution was collected and divided into two fractions (when the feed profiles were overlapping) in the following order: rhamnose fraction (containing most of the rhamnose) and xylose fraction (containing most of the xylose, other saccharides and salts). The sequential feeds can also be done without overlapping and thus the outcoming solution can be divided into four fractions in the following order: residual fraction number one (containing salts), rhamnose fraction (containing most of the rhamnose), xylose fraction (containing most of the xylose and some other monosaccharides) and residual fraction number two (containing other monosaccharides). Optionally between the outcoming fractions can be taken recycle fractions which can be recycled for diluting the feed or which can be fed as such into the column.

The amount of dry substance as well as rhamnose and xylose content in the feed solution and in product fractions are presented in Table 1. The concentrations of the successive components are expressed as percentages of the total dry substance in the particular fraction. The yield of rhamnose and xylose in product fractions are also presented (the amount of the component in the particular fraction in relation to the total amount of that component in all outcoming fractions).

Table1.

Compositions and yi lds (when profiles were overlapping and the outcoming solution was divided in two fractions)

	Feed solution	Rhamnose fraction	Xylose fraction
DS in fraction, kg	8,0	2,2	5,8
DS g/100 g solution	30,0	8,9	15,5
Rhamnose, % of DS in fraction	5,5	18,0	0,8
Xylose, % of DS in fraction	22,5	13,2	25,6
Rhamnose, yield %		90,0	10,0
Xylose, yield %		17,0	83,0

The pH of the effluent was between 7,3 and 9,3. The results are shown graphically in FIG. 2.

Example 3

Chromatographic separation of xylose-arabinose fraction from rhamnose separation

Arabinose containing xylose fraction, prepared as in Example 2, from rhamnose separation was subjected to a chromatographic separation. The separation was performed in a pilot chromatographic separation column as a batch process. The column with a diameter of 0,225 m was filled with a strong acid cation exchange resin (Finex CS 13 GC™, manufactured by Finex Oy, Finland). The height of the resin bed was 5,0 m. The cross-linkage degree of the resin was 5,5 % by weight DVB and the average particle size about 0,4 mm. The resin was in Ca^{2+} -form. A feeding device was placed at the top of the resin bed. The temperature of the column, feed solution and eluant water was approximately 65 °C. The flow rate in the column was adjusted to 30 l/h. A check filtration (through a filter bag) was made prior the separation.

The chromatographic separation was carried out as follows:

Step 1:

The dry substance of the feed solution was adjusted to 30 g dry substance in 100 g solution according to the refractive index (RI) of the solution:

Step 2:

15 litres of preheated feed solution was pumped to the top of the resin bed .

Step 3:

The feed solution was eluted downwards in the column by feeding preheated ion-exchanged water to the top of the column.

Step 4:

The density and conductivity of the outcoming solution were measured continuously. The outcoming solution was collected and divided into three fractions in the following order: residual fraction (containing some of the xylose), xylose rich fraction (containing most of the xylose and other monosaccharides) and arabinose rich fraction (containing most of the arabinose). The amount of dry substance as well as arabinose and xylose content in the feed solution and in product fractions are presented in table 2. The concentrations of the components are expressed as percentages of the total dry substance in the particular fraction. The yield of arabinose and xylose in product fractions is also presented (the amount of the component in the particular fraction in relation to the total amount of that component in all outcoming fractions).

Table 2. Compositions and yields

	Feed solution	Xylose fraction	Arabinose fraction
DS in fraction, kg	5,0	3,3	1,7
DS g/100 g solution	30		
Arabinose, %	3,7	0,5	10,0
Xylose, %	36,5	44,0	21,0
Arabinose, yield %		10,0	90,0
Xylose, yield %		80,0	20,0

Arabinose was eluting at the back slope of the profile. Galactose and mannose and especially glucose and xylose can be separated from arabinose effectively. The arabinose content (% of the total dry substance) in the arabinose rich product fraction was 3-fold compared to the arabinose content in feed solution and the arabinose recovery was then 90 %. The pH of the effluent is between 5,3 and 6. The results are shown graphically in FIG. 3.

Example 4

Chromatographic separation of xylose crystallization run-off with a Na^+ -form strong acid cation exchange resin

Xylose precipitation crystallization run-off which was birch wood based Ca-base si-cooking liquor was subjected to a chromatographic separation in a batch separation column. The separation was performed in a pilot scale chromatographic separation column as a batch process.

The whole equipment consisted of a feed tank, a feed pump, a heat exchanger, a chromatographic separation column, product containers, pipelines for input of feed solution as well as eluent water, pipelines for output and flow control for the outcoming liquid.

The column with a diameter of 0,225 m was filled with a strongly acid cation exchange resin (manufactured by Finex ltd, Finland). The height of the resin bed was approximately 5,1 m. The degree of cross-linkage was 5,5 % by weight DVB and the average particle size of the resin was 0,41 mm. The resin was regenerated into sodium (Na^+) form and a feeding device was placed at the top of the resin bed. The temperature of the column, feed solution and eluent water was approximately 65 °C. The flow rate in the column was adjusted to 30 l/h.

The feed solution was pretreated by filtration by using a pressure filter and diatomaceous earth as filter aid. The feed solution was then heated to 65 °C and the pH was adjusted to pH 6, after which the solution was filtered via filter.

Chromatographic separation was carried out as follows:

Step 1.

The dry substance of the feed solution was adjusted to 35 g dry substance in 100 g of solution according to the refractive index (RI) of the solution.

Step 2.

15 l of the preheated feed solution was pumped to the top of the resin bed.

Step 3.

The feed solution was eluted downwards in the column by feeding preheated ion-exchanged water to the top of the column.

Step 4.

The density and conductivity of the outcoming solution were measured continuously. The outcoming solution was collected and divided into two fractions in the following order:

residual fraction (containing most of the salts) and xylose fraction (containing xylose, rhamnose, arabinose and other monosaccharides).

The amount of dry substance as well as rhamnose, arabinose and xylose content in the feed solution and in product fraction (xylose fraction) are presented in table 3. The concentrations of the components are expressed as

percentages of the total dry substance in the particular fraction. The yield of rhamnose, arabinose and xylose in product fraction are also presented (the amount of the component in the particular fraction in relation to the total amount of that component in all outcoming fractions). The colour (ICUMSA, measured at pH 5) of the feed solution and product fraction are also presented as well as colour removal %.

Table 3.

Compositions, yields and colors

	Feed solution fraction (nominal)	Xylose fraction (analyzed from samples)	Residual (analyzed from samples)
DS in fraction, kg	5,9	4,3	2,1
DS g/100 g solution	34,5	9,3	3,5
Rhamnose, %	5,6	7,1	0,04
Arabinose, %	2,8	3,9	0,03
Xylose, %	26,0	37,7	0,1
Color, ICUMSA	38 900	5 000	
Rhamnose, yield %		99,7	
Arabinose, yield %		99,6	
Xylose, yield %		99,9	
Colour removal, %		87,1	

Most of the salts and color were removed from xylose precipitation crystallization run-off with a Na^+ -form strong acid cation exchange resin. Also the amounts of rhamnose, arabinose and xylose were higher in the product fraction than in the feed solution. The pH of the effluent was between 5,5 and 7,2. The results are shown graphically in FIG. 4.

Exampl 5

Chromatographic separation of rhamnose containing xylose fraction

Xylose fraction prepared according to example 4 (containing xylose, rhamnose, arabinose and other monosaccharides) was subjected to a chromatographic separation in a batch separation column. The separation was performed in a pilot scale chromatographic separation column as a batch process.

The whole equipment consisted of a feed tank, a feed pump, a heat exchanger, a chromatographic separation column, product containers, pipelines for input of feed solution as well as eluent water, pipelines for output and flow control for the outcoming liquid.

The column with a diameter of 1,0 m was filled with a weakly acid cation exchange resin (Finex CA 16 GC™) manufactured by Finex Ltd, Finland. The resin was methyl acrylate –based resin. The height of the resin bed was approximately 5,0 m. The degree of cross-linkage was 8 % by weight DVB and the average particle size of the resin was 0,28 mm. The resin was regenerated into sodium (Na^+) form and a feeding device was placed at the top of the resin bed. The temperature of the column, feed solution and eluent water was 65°C. The flow rate in the column was adjusted to 785 l/h.

The pH of the feed solution was adjusted to pH 6,5 after which the solution was filtered via filter.

Chromatographic separation was carried out as follows:

Step 1.

The dry substance of the feed solution was adjusted to 35 g dry substance in 100 g of solution according to the refractive index (RI) of the solution.

Step 2.

400 l of the preheated feed solution was pumped to the top of the resin bed.

Step 3.

The feed solution was eluted downwards in the column by feeding preheated ion-exchanged water to the top of the column.

Step 4.

The density and conductivity of the outcoming solution were measured continuously. The outcoming solution was collected and divided into three fractions (when the feed profiles were not overlapping) in the following order: residual fraction (containing most of the salts), rhamnose rich fraction (containing most of the rhamnose) and xylose rich fraction (containing most of the xylose, arabinose and other monosaccharides).

The amount of dry substance as well as rhamnose and xylose content in the feed solution and in product fractions are presented in table 4. The concentrations of the components are expressed as percentages of the total dry substance in the particular fraction. The yield of rhamnose and xylose in product fractions is also presented (the amount of the component in the particular fraction in relation to the total amount of that component in all outcoming fractions).

Table 4. Compositions and yields

	Feed solution	Rhamnose fraction	Xylose fraction
DS in fraction, kg	160	44	114
DS g/100 g solution	36,1	6,2	10,6
Rhamnose, %	6,7	21,9	0,9
Xylose, %	37,4	24,5	36,5
Rhamnose, yield %		90,4	–
Xylose, yield %		--	79,0

Rhamnose content (% of the total dry substance) in rhamnose rich product fraction was 3,3-fold compared to rhamnose content in feed solution.

Rhamnose was separated from feed solution with a good yield. The pH of the effluent was between 8 and 9. The reusults are shown graphically in FIG. 5. Arabinose can be separated from the xylose fraction for example by using a strongly acid cation exchange resin.

Example 6

Chromatographic separation of rhamnose rich fraction with a weakly acid cation exhange resin

Rhamnose rich fraction prepared according to example 5 was subjected to a chromatographic separation in a batch separation column. The separation was performed in a pilot scale chromatographic separation column as a batch process.

The whole equipment consisted of a feed tank, a feed pump, a heat exchanger, a chromatographic separation column, product containers, pipelines for input of feed solution as well as eluent water, pipelines for output and flow control for the outcoming liquid.

The column with a diameter of 1,0 m was filled with a weakly acid cation exchange resin (Finex CA 16 GC™) manufactured by Finex Ltd, Finland. The resin was methyl acrylate –based resin. The height of the resin bed was approximately 5,0 m. The degree of cross-linkage was 8 w-% DVB and the average particle size of the resin was 0,28 mm. The resin was regenerated into sodium (Na^+) form and a feeding device was placed at the top of the resin bed. The temperature of the column, feed solution and eluent water was 65°C. The flow rate in the column was adjusted to 785 l/h.

The pH of the feed solution was adjusted to pH 6,5 after which the solution was filtered via filter.

Chromatographic separation was carried out as follows:

Step 1.

The dry substance of the feed solution was adjusted to 35 g dry substance in 100 g of solution according to the refractive index (RI) of the solution.

Step 2.

250 l of the preheated fe d solution was pumped to the top of the resin bed.

Step 3.

The feed solution was eluted downwards in the column by feeding preheated ion-exchanged water to the top of the column.

Step 4.

The density and conductivity of the outcoming solution were measured continuously. The outcoming solution was collected and divided into three fractions (when the feed profiles were not overlapping) in the following order: first residual fraction (containing most of the salts), rhamnose rich frac- tion (containing most of the rhamnose) and second residual fraction (containing most of the xylose and other monosaccharides).

The amount of dry substance as well as rhamnose and xylose content in the feed solution and in product fraction are presented in table 5. The concentrations of the components are expressed as percentages of the total dry substance in the particular fraction. The yield of rhamnose in product fraction is also presented (the amount of the component in the particular fraction in relation to the total amount of that component in all outcoming fractions).

Table 5. Compositions and yields

	Feed solution	Rhamnose fraction
DS in fraction, kg	100	39
DS g/100 g solution	35,5	8,6
Rhamnose, %	21,6	47,0
Xylose, %	23,1	6,2
Rhamnose, yield %		86,0

The rhamnose content (% of the total dry substance) in product fraction was 2,2-fold compared to rhamnose content in feed solution. Rhamnose was separated from feed solution with a good yield. The pH of the effluent was between 8 and 10. The results are shown graphically in FIG. 6.

Example 7

Chromatographic separation of rhamnose rich fraction with a Ca^{2+} -form strong acid cation exchange resin

Rhamnose rich fraction prepared according to example 6 was subjected to a chromatographic separation in a batch separation column. The separation was performed in a pilot scale chromatographic separation column as a batch process.

The whole equipment consisted of a feed tank, a feed pump, a heat exchanger, a chromatographic separation column, product containers, pipelines for input of feed solution as well as eluent water, pipelines for output and flow control for the outcoming liquid.

The column with a diameter of 0,6 m was filled with a strong acid cation exchange resin (Finex CS 11 GC) manufactured by Finex Ltd, Finland. The height of the resin bed was approximately 5,0 m. The degree of cross-linkage was 5,5 w-% DVB and the average particle size of the resin was 0,40 mm. The resin was regenerated into sodium (Ca^{2+}) form and a feeding device was placed at the top of the resin bed. The temperature of the column, feed solution and eluent water was 65 °C. The flow rate in the column was adjusted to 210 l/h.

The pH of the feed solution was adjusted to pH 6,5 after which the solution was filtered via filter.

Chromatographic separation was carried out as follows:

Step 1.

The dry substance of the feed solution was adjusted to 30 g dry substance in 100 g of solution according to the refractive index (RI) of the solution.

Step 2.

1101 of the preheated feed solution was pumped to the top of the resin bed.

Step 3.

The feed solution was eluted downwards in the column by feeding preheated ion-exchanged water to the top of the column.

Step 4.

The density and conductivity of the outcoming solution were measured continuously. The outcoming solution was collected and divided into three fractions (when the feed profiles were not overlapping) in the following order: first residual fraction (containing components other than monosaccharides), rhamnose rich fraction (containing most of the rhamnose) and second residual fraction (containing other monosaccharides and other components).

The amount of dry substance as well as rhamnose content in the feed solution and in product fraction are presented in table 6. The concentration of rhamnose is expressed as percentage of the total dry substance in the particular fraction. The yield of rhamnose in product fraction is also presented (the amount of the component in the particular fraction in relation to the total amount of that component in all outcoming fractions).

Table 6. Compositions and yields

	Feed solution (nominal)	Rhamnose fraction (analyzed from samples)
DS in fraction, kg	37	34,8
DS g/100 g solution	30	10,2
Rhamnose, %	47,9	55,4
Rhamnose, yield %		99,0

Rhamnose purity was increased by 16 %. Rhamnose yield was excellent being 99 %. The pH of the effluent was between 3,5 and 4. The results are shown graphically in FIG. 7.

Example 8

Crystallization of rhamnose

13100 g of a rhamnose syrup having DS of 14 % and a rhamnose content of 52,3 %, based on the refractometric dry solids content of pure rhamnose, was evaporated to RDS of 86,9 % and moved to a 2-liter reaction vessel at a temperature of 65 °C. Seeding (at 65 °C, a RDS of 86,9 %) was made to the boiling syrup with 0,03 % seeds on DS.

The mass was cooled down from a temperature of 65 °C to a temperature of 40 °C in 16 hours. After 16 hours from seeding, the centrifuging without wash gave a cake purity 98,5 % on RDS and a mother liquor purity 21,2 % on RDS, which corresponds to a 76 % rhamnose yield. The crystal size was 200... 350 µm. The moisture content of the dried crystals was 10,0 % measured with a Karl Fischer titration method.

The results are shown in table 7.

Table 7

Sample	RDS %	Monosaccharides (PED LC) % on weight RDS						
	by weight	Rhamnose	Arabinose	Galactose	Glucose	Xylose	Mannose	Fructose
Feed	14,1	52,3	0,6	4,6	2,3	12,8	4,6	0,6
Mother liquor, no wash	54,3 *	21,2	0,7	7,8	4,0	21,3	7,8	1,0
Cake, no wash	90,0	98,5	-	0,1	0,0	0,3	0,1	-

* diluted sample

Exampl 9

Arabinose crystallization

The arabinose containing feed liquid was added to a 400-litre boiling crystallizer. The evaporation was started at a temperature of 60 °C and at a pressure of 10 mbar. The boiling liquid was seeded with 0,03 % seeds on DS at DS of 67,9 % at a temperature of 60 °C and at a pressure of 130 mbar. After seeding the boiling crystallization was continued for 3 hours at a temperature of 60 °C and a new feed liquid was added continuously into the boiling crystallizer. A 400-litre batch of the mass obtained by boiling crystallization (DS of mass 68,9 %) was moved to a 400-litre cooling crystallizer.

The mass was cooled down from a temperature of 60 °C to a temperature of 30 °C in 20 hours. After cooling crystallization the mass was centrifuged. The crystals were dried and packed.

What is claimed is:

1. A method for recovering a monosaccharide selected from the group consisting of rhamnose, arabinose, xylose and mixtures thereof from a solution containing at least two of said monosaccharides by a multistep process using chromatographic separation comprising at least one step, where a weakly acid cation exchange resin is used for the chromatographic separation.

2. The method of claim 1 comprising feeding the solution containig a monosaccharide selected from the group consisting from rhamnose, arabinose, xylose and mixtures thereof into a chromatographic column containing a weakly acid cation exchange resin, eluting said column with an eluant, and separating and recovering the rhamnose fraction.

3. The method of claim 1 or 2 wherein also a strongly acid cation exchange resin is used in a chromatographic column.

4. The method of any of claims 1 to 3 wherin the multistep process further comprises steps selected from the group consisting of crystallization, filtration, evaporation, precipitation and ion exchange.

5. The method of any of claims 1 to 4 where the monosaccharide recovered is rhamnose.

6.The method of any of claim 5 wherein the rhamnose recovered is L-rhamnose.

7. The method of any of claims 1 to 6 wherein the solution containing rhamnose is a xylose process stream or side stream.

8. The method of any of claims 5 to 7 wherein a arabinose rich fraction is separated and recovered.

9. The method of claim 8 wherein the arabinose to be recovered is L-arabinose.

10. The method of any of claims 5 to 9 wherein a xylose rich fraction is separated and recovered.

11. The method of claim 10 wherein the xylose to be recovered is D-xylose.

12. The method of any of claims 1 to 11 wherein the weakly acid cation exchange resin is an acrylic resin.

13. The method of claim 12 wherein the acrylic resin is derived from the group consisting of methyl acrylate, ethyl acrylate, buthyl acrylate methyl methacrylate and acrylonitrile and acrylic acids and mixtures thereof.

14. The method of claim 13 wherein the resin is in the form selected from the group consisting of Na^+, Mg^{2+}, H^+ and Ca^{2+}.

15. The method of claim 14 wherein the resin is in Na^+ form.

16. The method of any of claims 12 to 15 wherein the resin is crosslinked with divinyl benzene (DVB).

17. The method of claim 16 wherein the crosslinking degree of the resin is 3 to 8 % by weight.

18. The method of any of claims 1 to 17 wherein the eluent is water.

19. The method of any of claims 1 to 18 comprising feeding the solution containing rhamnose to a first chromatographic column and then feeding a fraction from the first chromatographic column to a second chromatographic column, both columns containing a weakly acid cation exchange resin.

20. The method of claim 19 comprising feeding a fraction from the second chromatographic column to a third chromatographic column containing a strongly acid cation exchange resin and feeding a fraction from the third chromatographic column to a fourth chromatographic column containing strongly acid cation exchange resin.

21. The method of any of claims 1 to 18 comprising feeding the solution containig rhamnose to a first chromatographic column containing a strongly acid cation exchange resin and then feeding a fraction from the first chromatographic column to a second chromatographic column containing a weakly acid cation exchange resin.

22. The method of claim 21 comprising feeding a fraction from the second chromatographic column to a third chromatographic column containing a weakly acid cation exchange resin.

23. The method of claim 19 or 21 comprising feeding a fraction from the second chromatographic column to a third chromatographic column containing a strongly acid cation exchange resin.

24. The method of any of claims 19 to 23 wherein prior to feeding the fraction to the next chromatographic column said fraction is concentrated by evaporation.

25. The method of any of claims 1 to 24 wherein the temperature of the eluant is between 10 °C and 95 °C.

26. The method of claim 25 wherein the temperature of the eluent is between 55 °C and 85 °C.

27. The method of any of claims 1 to 26 wherein the particle size of the weakly acid cation exchange resin is 10 to 2000 μm.

28. The method of claim 27 wherein the particle size of the weakly acid cation exchange resin is 100 to 400 μm.

29. The method of any of claims 1 to 28 wherein the pH of the feed solution is 1 to 10.

30. The method of claim 29 wherein the pH of the feed solution is 2 to 4.

31. The method of claim 29 wherein the pH of the feed solution is 5 to 10.

32. The method of any of claims 19 to 31 comprising recovering from the first and the second chromatographic column xylose and arabinose.

33. The method of any of claims 1 to 32 comprising isolating arabinose by crystallization.

34. The method of any of claims 19 to 33 comprising recovering rhamnose from the second and/or the third chromatographic column.

35. The method of any of claims 1 to 34 comprising isolating L-rhamnose by crystallization.

36. The method of any of claims 1 to 35 comprising isolating L-rhamnose in the form of monohydrate.

37. The method of any of claims 1 to 36 comprising isolating xylose by crystallization.

38. The method of any of claims 1 to 37 wherein the method is a batch process.

39. The method of any of claims 1 to 38 wherein the rhamnose fraction is collected before the other saccharides.

40. The method of any of claims 1 to 38 wherein rhamnose fraction is collected after the other sacharides.

41. The method of any of claims 1 to 40 wherein rhamnose and arabinose are collected together.

42. The method of any of claims 1 to 41 wherein the chromatographic separation method is a simulated moving bed system.

43. The method of claim 42 wherein the simulated moving bed system is sequential.

44. The method of claim 43 wherein the simulated moving bed system is continuous.

45.The method of any of claims 42 to 44 wherein at least one column or a part of a column contains a strongly acid cation exchange resin and at least one column contains a weakly acid cation exchange resin.

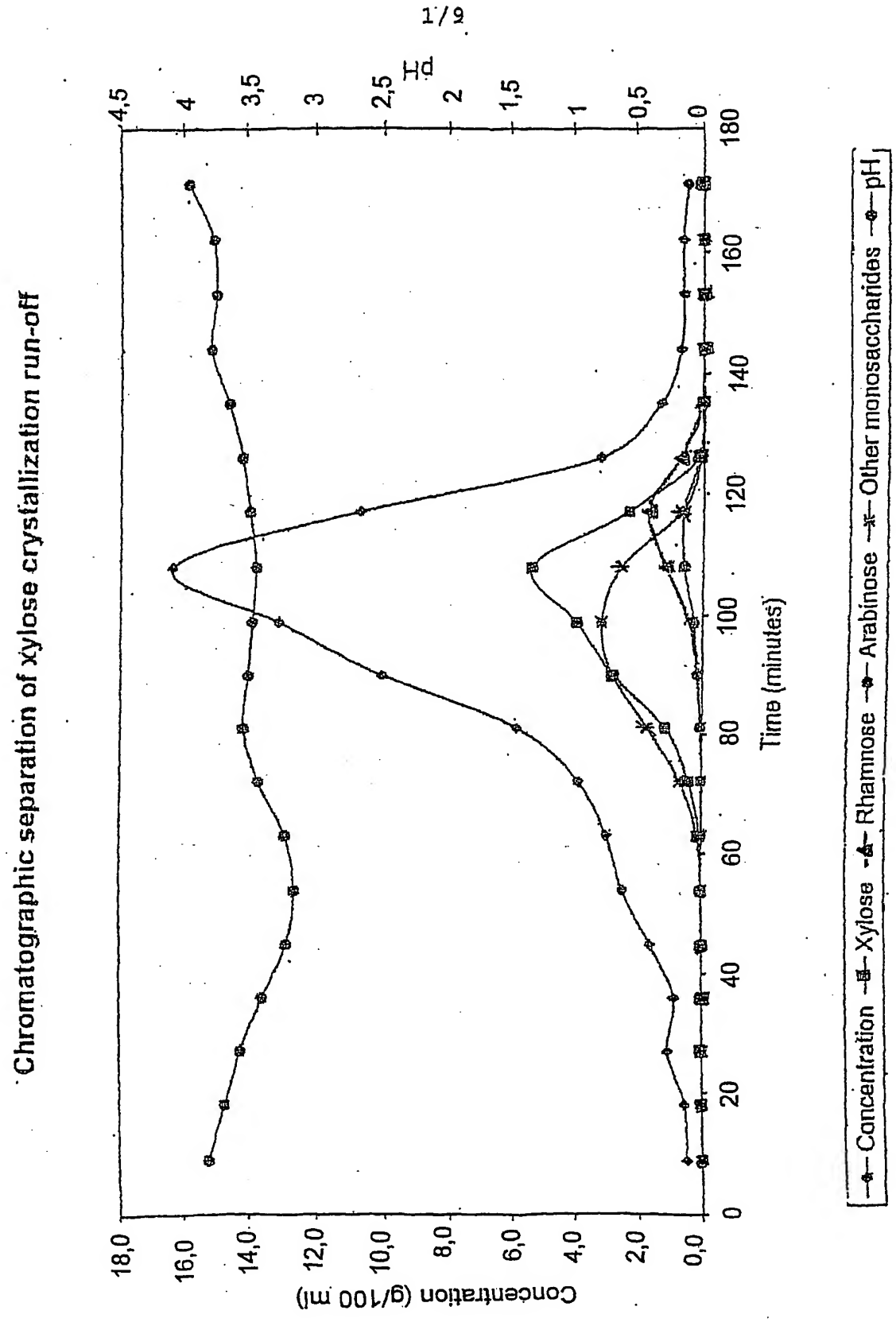

Chromatographic separation of xylose crystallization run-off
Concentration (g/100 ml)
pH
Time (minutes)
Concentration
Xylose
Rhamnose
Arabinose
Other monosaccharides
pH

FIG. 1

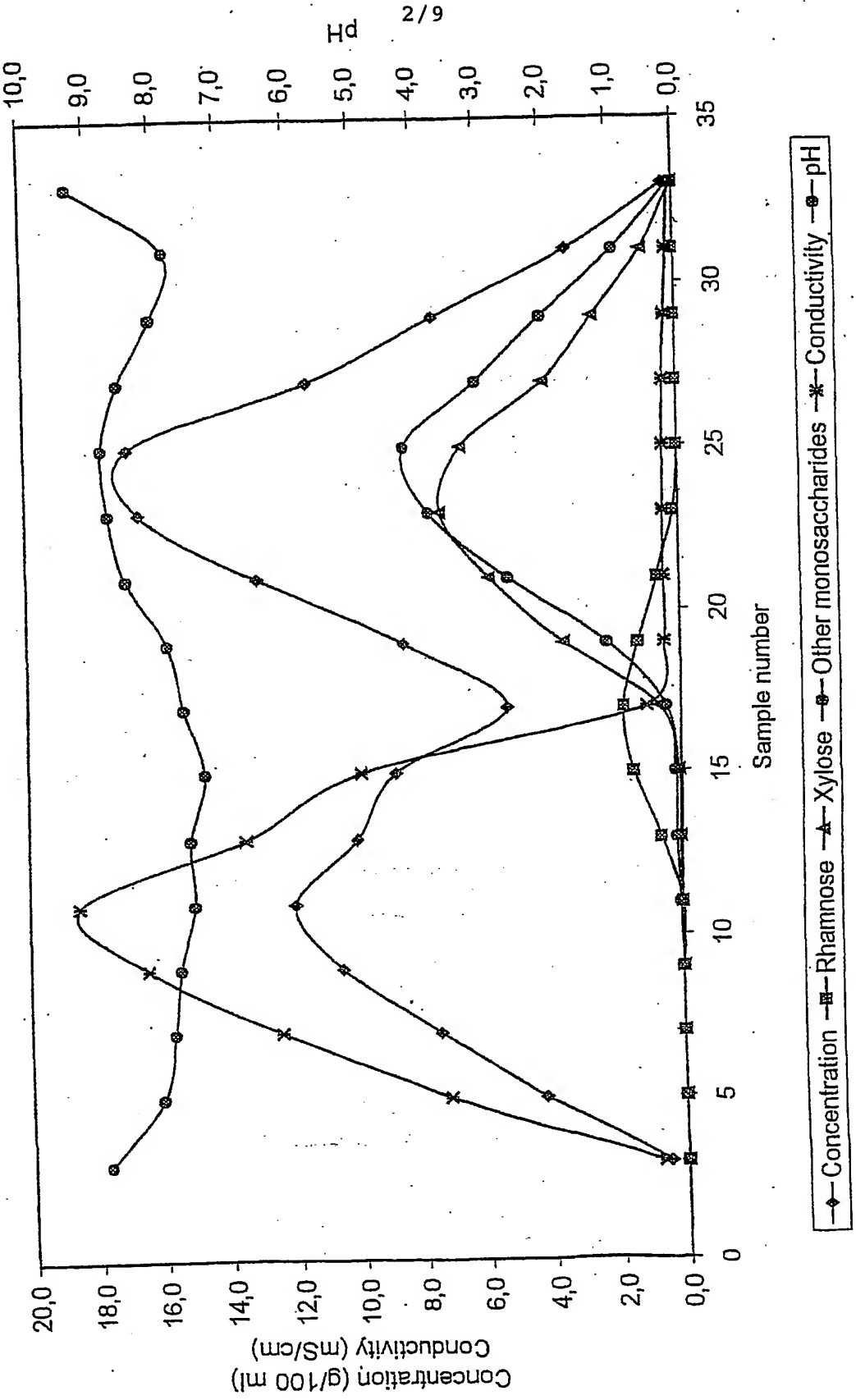
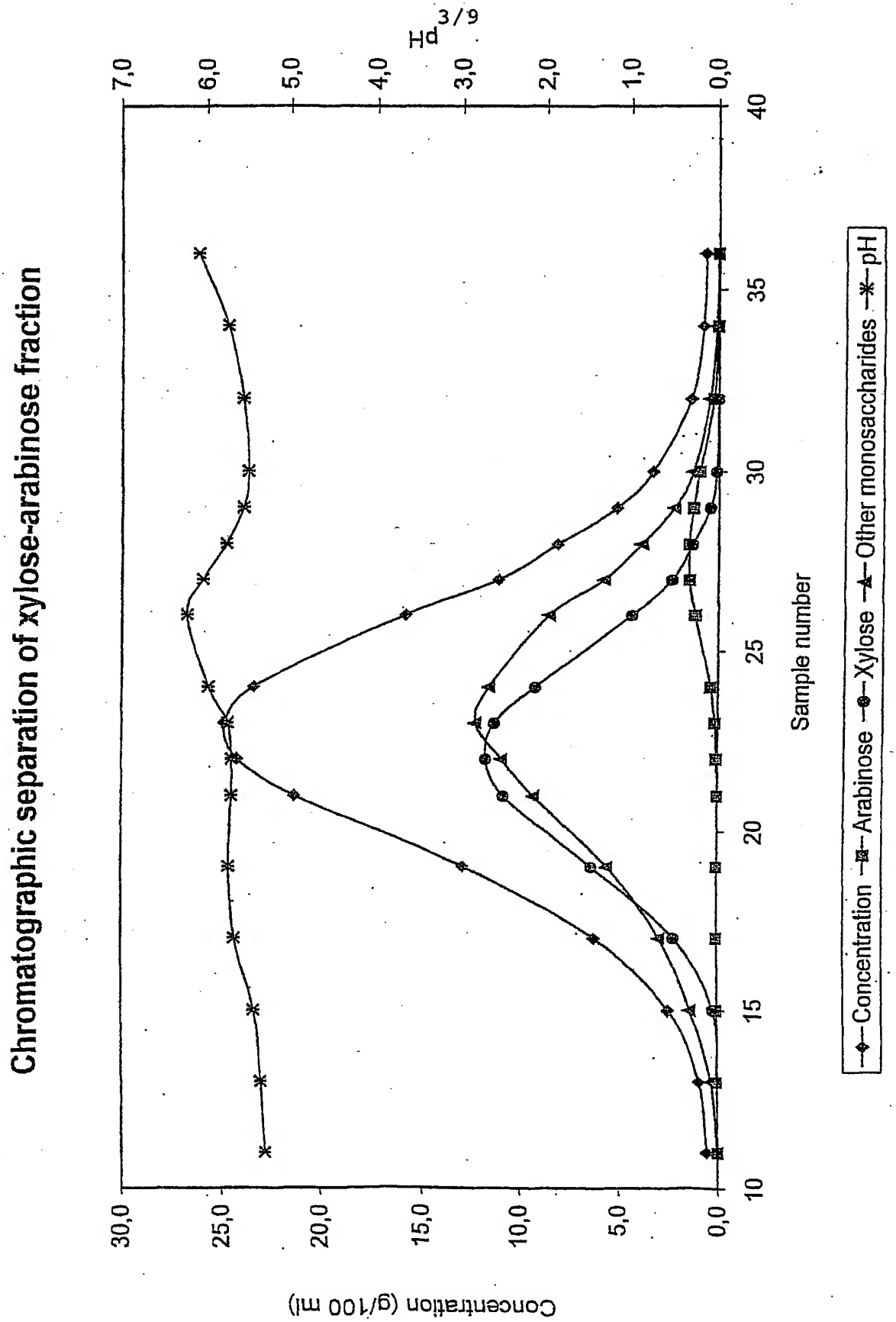

Chromatographic separation of xylose crystallization run-off
Concentration (g/100 ml)
Conductivity (mS/cm)
pH
Sample number
0,0
2,0
4,0
6,0
8,0
10,0
12,0
14,0
16,0
18,0
20,0
1,0
3,0
5,0
7,0
9,0
0
5
10
15
20
25
30
35
Concentration
Rhamnose
Xylose
Other monosaccharides
Conductivity
pH

FIG. 2


Chromatographic separation of xylose-arabinose fraction
Concentration (g/100 ml)
pH
Sample number
Concentration
Arabinose
Xylose
Other monosaccharides
pH
30,0
25,0
20,0
15,0
10,0
5,0
0,0
7,0
6,0
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10
15
20
25
30
35
40

FIG. 3

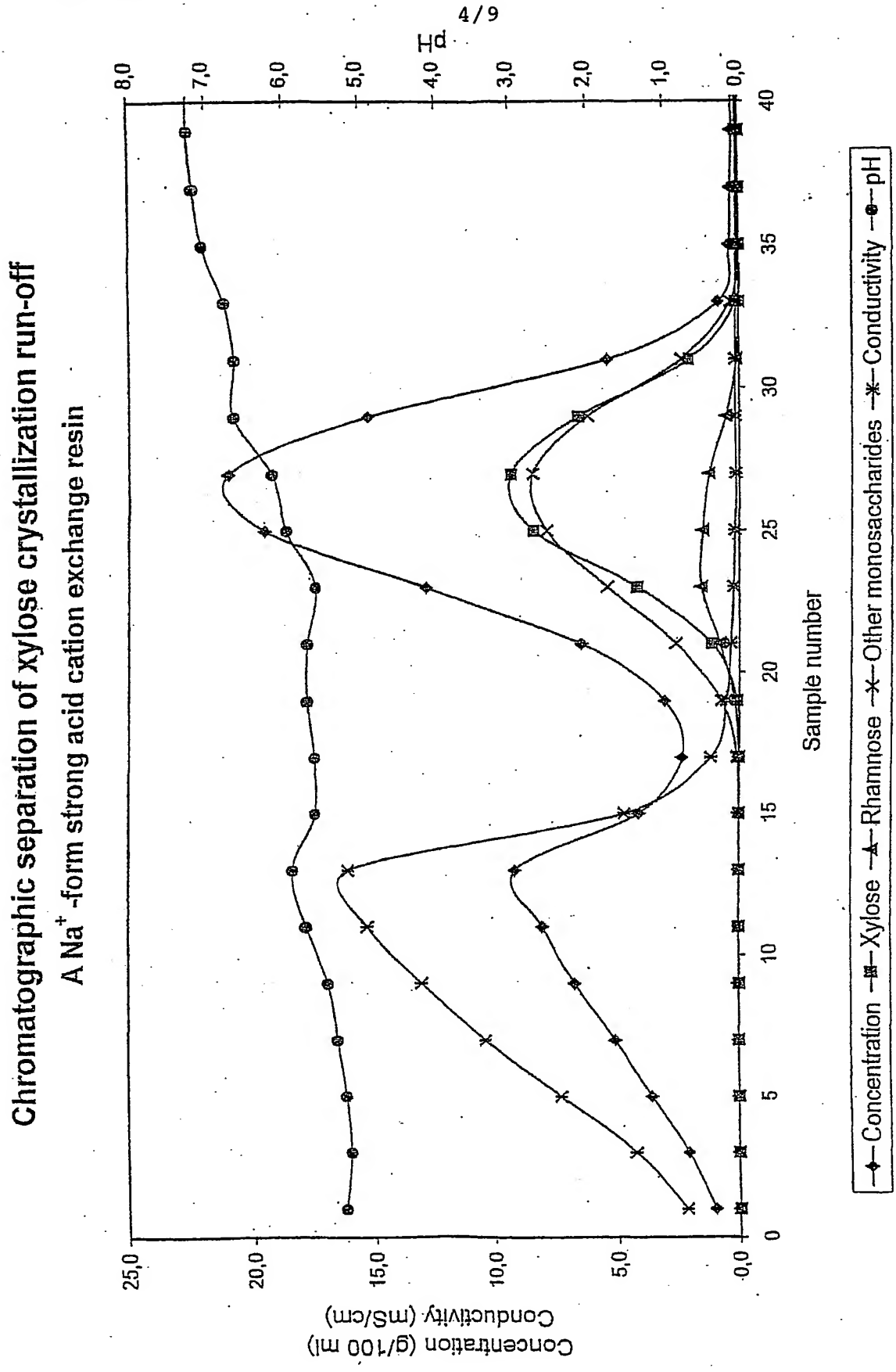
Chromatographic separation of xylose crystallization run-off
A Na+ -form strong acid cation exchange resin
Concentration (g/100 ml)
Conductivity (mS/cm)
pH
Sample number
Concentration
Xylose
Rhamnose
Other monosaccharides
Conductivity
pH

FIG. 4

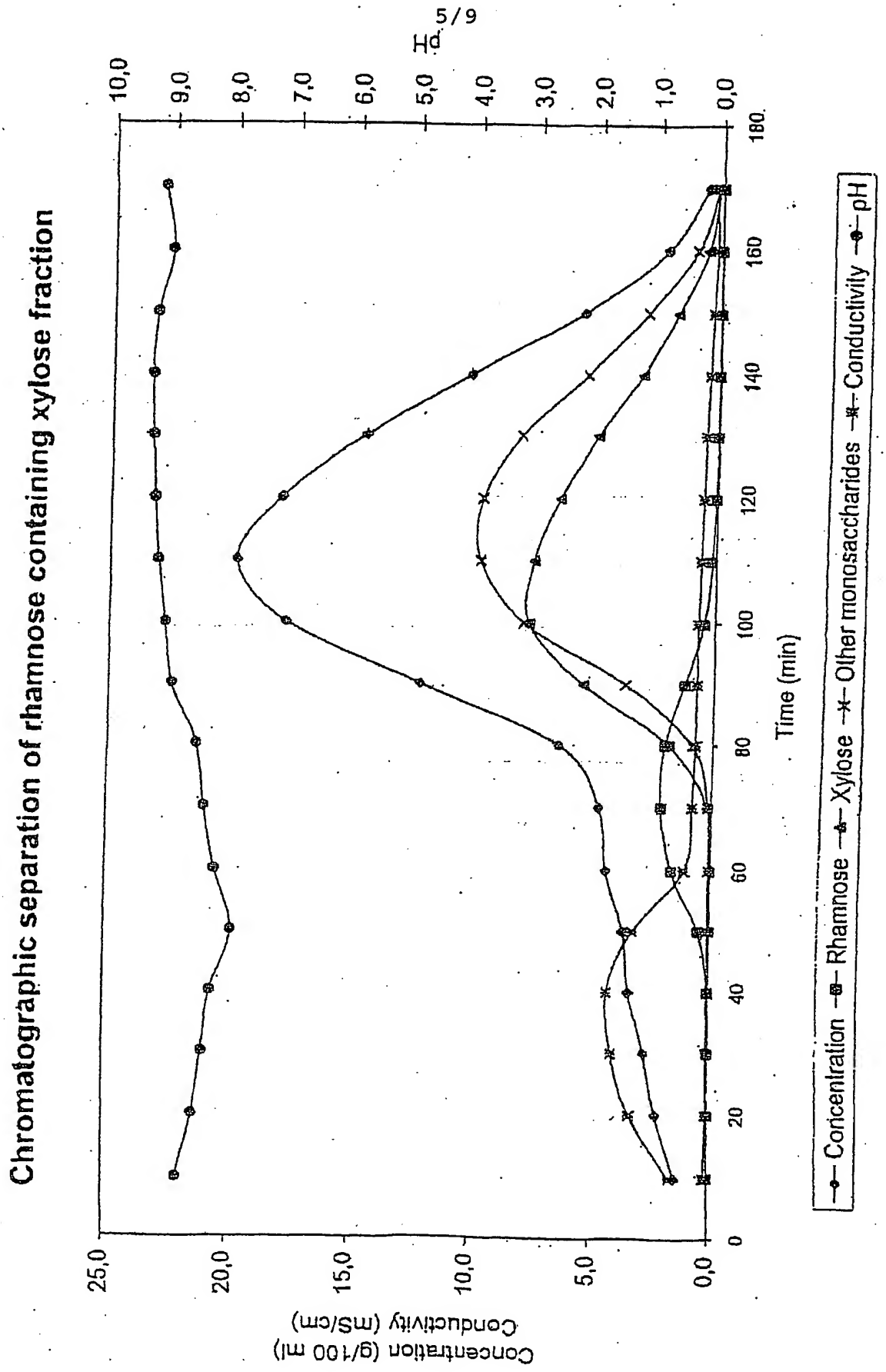
Chromatographic separation of rhamnose containing xylose fraction
Concentration (g/100 ml)
Conductivity (mS/cm)
pH
Time (min)
Coricenlration
Rhamnose
Xylose
Other monosaccharides
Conductivity
pH

FIG. 5

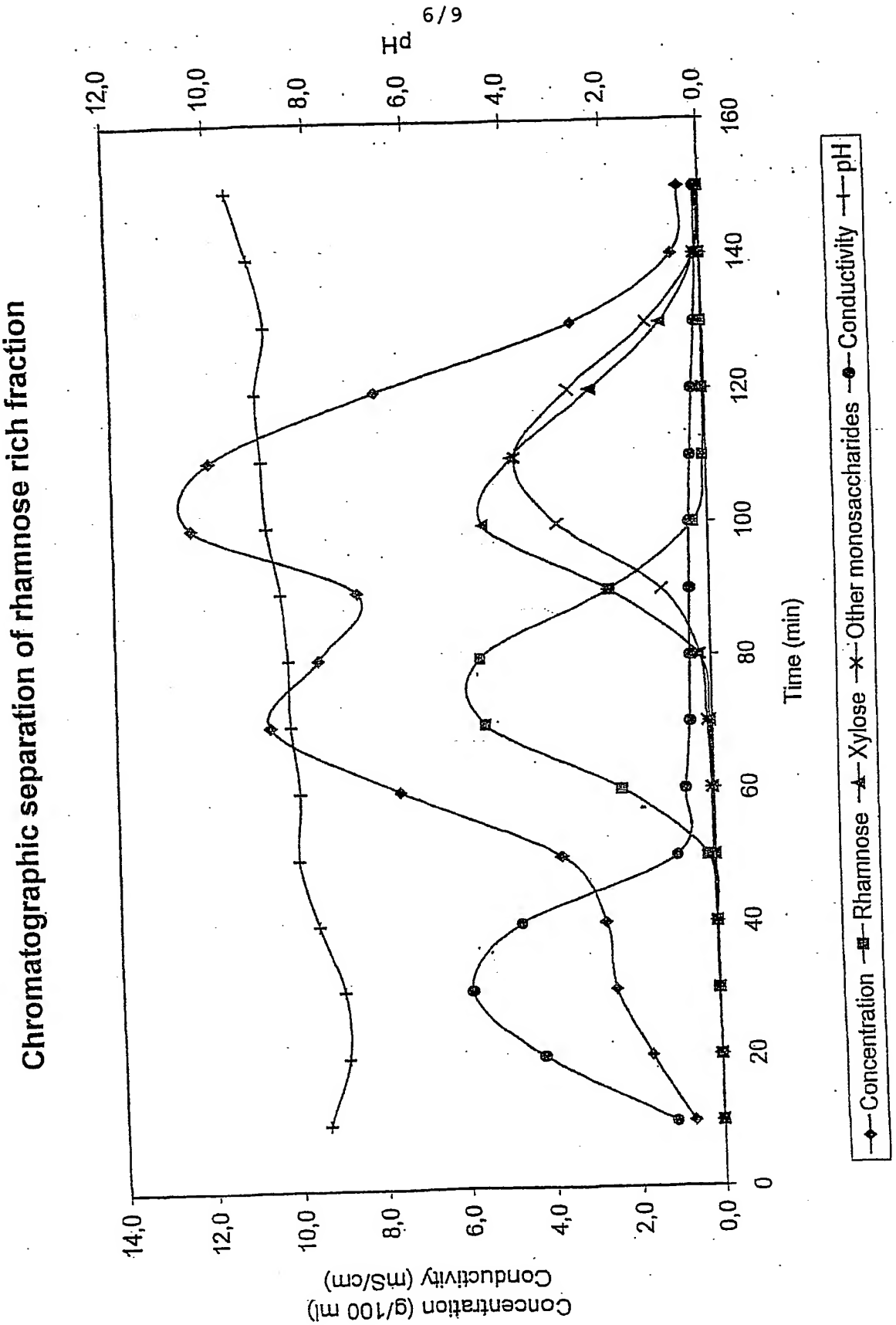
Chromatographic separation of rhamnose rich fraction
Concentration (g/100 ml)
Conductivity (mS/cm)
pH
Time (min)
0,0
2,0
4,0
6,0
8,0
10,0
12,0
14,0
0
20
40
60
80
100
120
140
160
Concentration
Rhamnose
Xylose
Other monosaccharides
Conductivity
pH

FIG. 6

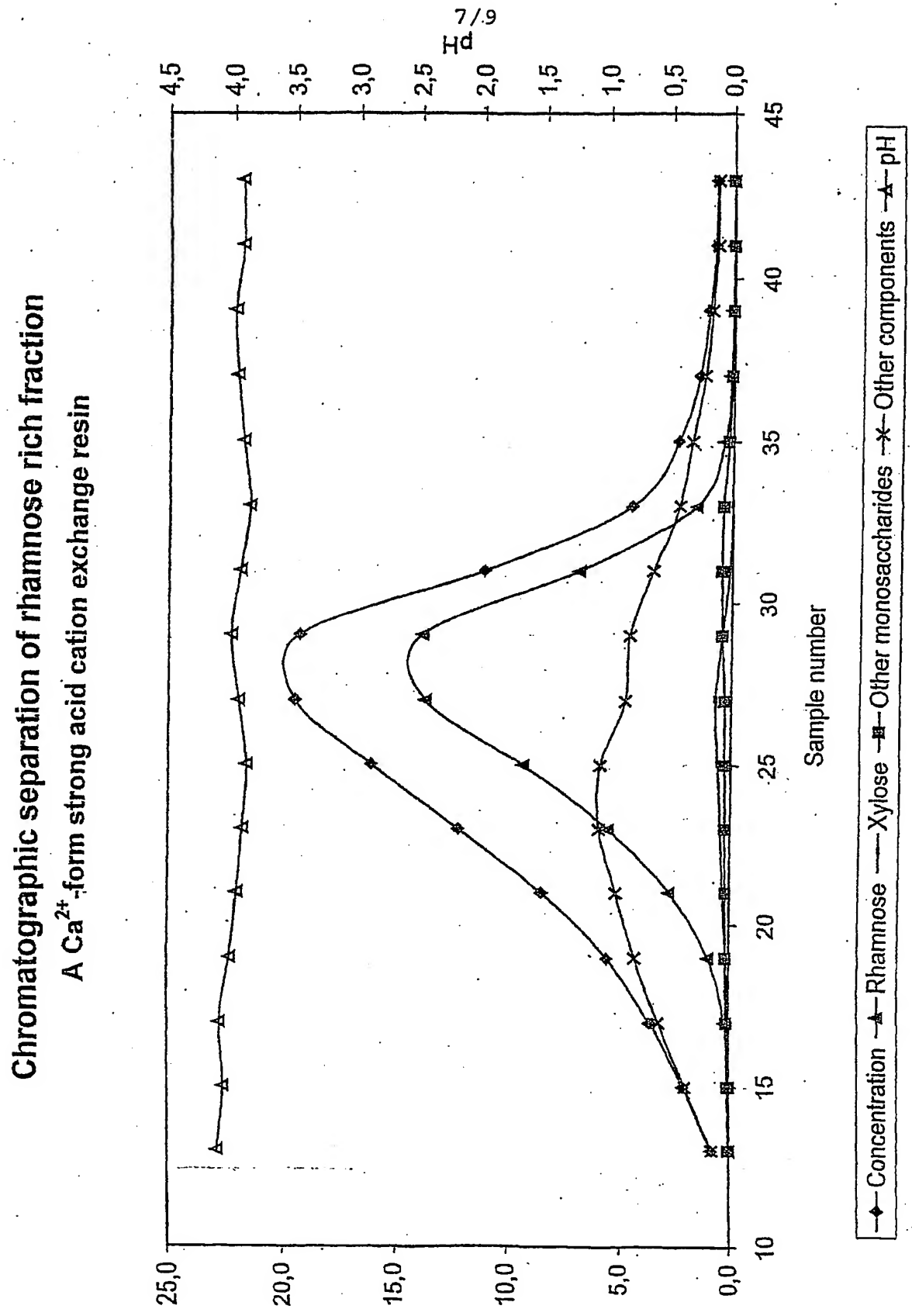
Chromatographic separation of rhamnose rich fraction
A Ca^{2+}-form strong acid cation exchange resin
Concentration (g/100 ml)
pH
Sample number
Concentration
Rhamnose
Xylose
Other monosaccharides
Other components
pH

FIG. 7

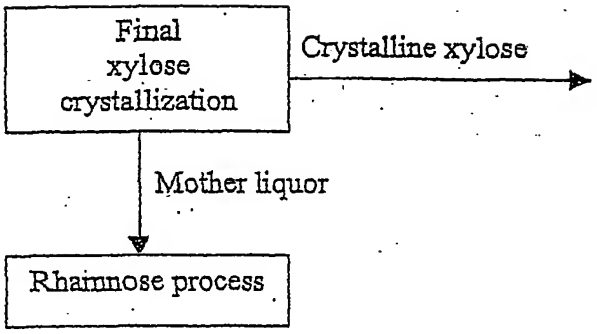
Final
xylose
crystallization
Crystalline xylose
Mother liquor
Rhamnose process

FIG. 8

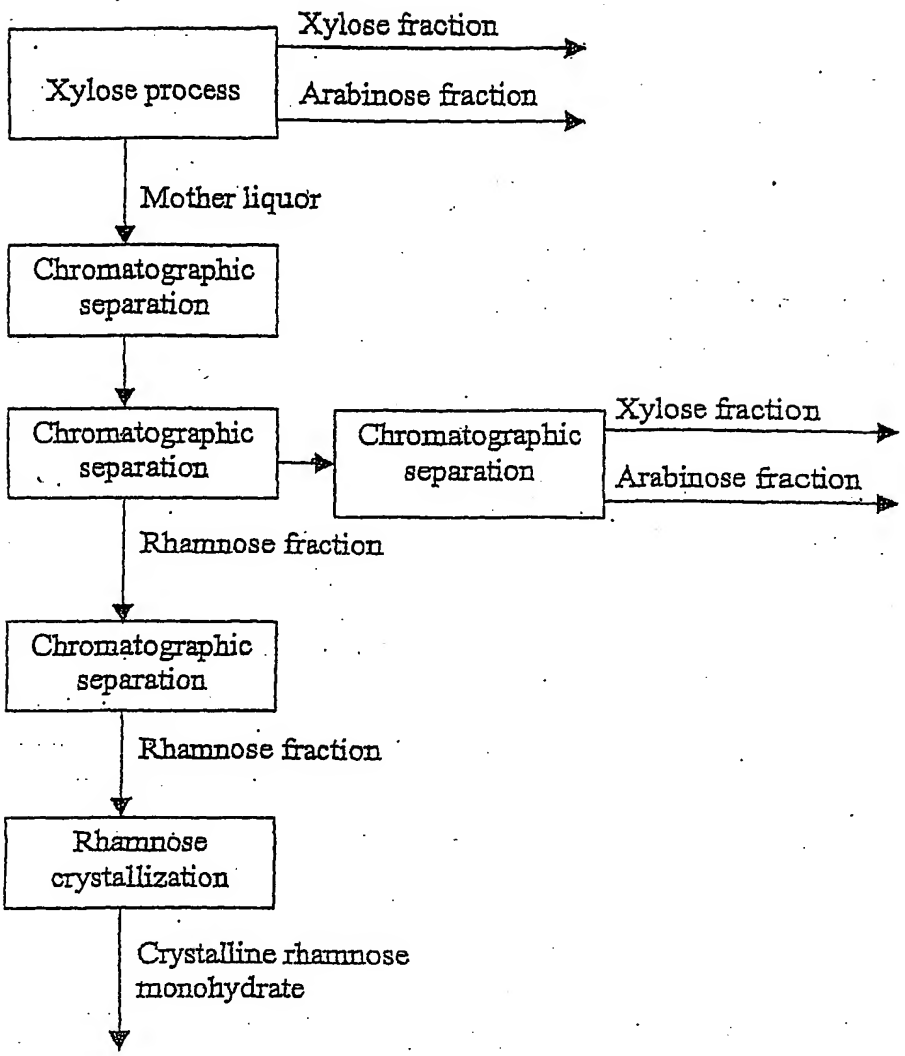
Xylose process
Xylose fraction
Arabinose fraction
Mother liquor
Chromatographic separation
Chromatographic separation
Chromatographic separation
Xylose fraction
Arabinose fraction
Rhamnose fraction
Chromatographic separation
Rhamnose fraction
Rhamnose crystallization
Crystalline rhamnose monohydrate

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 01/00848

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C13K 13/00, C13D 3/14, C13J 1/06
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C13K, C13D, C13J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5998607 A (HEIKKI HEIKKILÄ ET AL), 7 December 1999 (07.12.99), exampels 10, 11 --	1-45
X	Journal of Chromatography, Volume 256, 1983, Wolfgang Blaschek: "Complete separation and quantification of neutral sugars from plant cell walls and mucilages by high-performance liquid chromatography", page 157 - page 163 --	1-45
X	WPI/Derwent's abstract, Accession Number 2000-376948, week 0033, ABSTRACT OF CN, 1234404 (UNIV HUADONG SCI & ENG) 10 November 1999 (10.11.99) --	1-45

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 February 2002	08 -02- 2002
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Anna Sjölund/EÖ Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 01/00848

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA on CD, Accession no. 127:47442, Oishi, Kazuyuki et al: "Liquid column chromatography packed with two media for efficient separation of sugars", Jpn. Kokai Tokkyo Koho JP 09127090 A2 16 May 1997 Heisei, 5pp (Japan) --	1-45
X	STN International, File CAPLUS, CAPLUS accession no. 1982:541125, Document no. 97.141125, Trusova, L. I. et al: "Chromatographic determination of carbohydrates and ketonic acids in culture media"; Tr. - Vses. Gos. Nauchno-Kontrol'n. Inst. Vet. Prep., 29-30, 114-21 (Russian) 1980 -- --------	1-45

INTERNATIONAL SEARCH REPORT

Information on patent family members

27/12/02

International application No.
PCT/FI 01/00848

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5998607 A	07/12/99	AU 3178997 A	14/01/98
		CA 2258866 A	31/12/97
		CZ 9804090 A	14/04/99
		EP 0914312 A	12/05/99
		FI 102962 B	00/00/00
		FI 962610 A	25/12/97
		JP 2000511199 T	29/08/00
		SK 175998 A	11/06/99
		WO 9749659 A	31/12/97